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THE ESSENTIAL NATURE OF CERTAIN MINOR ELEMENTS FOR PLANT NUTRITION

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Until about the beginning of this century, ten or eleven elements only were generally considered as essential for normal growth of plants. The presence of other elements in minute quantities was recognized in many plants, but their possible association with nutrition and growth was not understood. The activities of the French investigators from 1897 onwards focussed attention on the function of these minimal traces of elements, and the work of Bertrand (9) on manganese, Javillier (70) on zinc and Agulhon (1) on boron may be regarded as the foundation of the widespread investigations which are now of such practical economic importance.

The French school claimed that some of these minor elements were essential for the full development of certain plants, but the experimental difficulties in obtaining adequate proof were not at that time fully overcome, and for a time the matter remained of academic, rather than practical, importance. Still, interest had been awakened, and much experimental work resulted in many and varied claims being made for different elements—claims which frequently were not borne out by other tests under different conditions. In 1923, however, Warington (160) was able to prove conclusively that a trace of boron is absolutely essential for the development of *Vicia faba,* as in its absence the meristematic tissues die and growth is impossible. Since then, investigations all over the world indicate that boron is probably essential for all plants in varying degree and that certain obscure plant diseases may be due to a deficiency of this element.

Although it is more difficult to get clear-cut proof of the essential nature of manganese for all plants, the whole body of evidence

is now so strong that this statement is generally accepted as fact. The small amount that is necessary and the closeness of the association of manganese with iron render this element peculiarly difficult for experimental treatment. Copper and zinc have also attracted much attention and their value in certain cases seems evident, though they cannot lay claim to the importance of manganese and boron.

Work has also been done with a wide range of other elements, often with conflicting results and in no case affording proof of their universal value. Stimulation of growth is frequently recorded, but up to the present most of the information gained deals with the relative toxicity of larger amounts of the elements. It must not be forgotten, however, that there is a possibility that certain elements may prove to be essential for certain plants, though not for all. If this is the case, the proof of the association of the particular element and plant will be difficult to obtain by deliberate investigation, but is more likely to be the result of a fortunate chance observation.

During the last thirty-five years the literature on the relation of minor elements to plants has become very extensive. Willis' (165) bibliography covers between 2000 and 3000 references with abstracts, and Jacks and Scherbatoff (69) give more than 350 others, but even so, the whole field is not covered. Much of this literature deals with the toxic and fungicidal aspects of the subject, but in the present review attention is chiefly confined to work done on the possible essential nature of certain elements for plant growth during the last five years. In spite of this narrowing of the subject, it is almost invidious to select certain papers for comment and to omit others, and such omission in no way implies that any particular piece of work is of less value than those actually quoted. Anyone who is interested in any particular element is strongly recommended to refer to the two bibliographies above mentioned in order to get a more adequate idea of the available information.

BORON

The essential nature of boron for many species of plants was fully established before 1930, and the possible connection of boron deficiency with certain plant diseases was already under consideration, as in the extensive investigations of Mes on "topsiekte" in

tobacco (102). During the last five years the practical aspect of the matter has been widely investigated, and boron deficiency is now suspected to be the cause of a variety of obscure physiological diseases which cannot be traced to insect or fungus attack.

Heart-rot, crown-rot, or dry-rot of sugar beet is characterized by the blackening and death of the central leaves, coupled with discoloration or rotting of the upper part of the root. Late in the season secondary growing points develop numerous small leaves, giving the plants a very dense, short, green top. Where the disease is prevalent, the yield is often very low and the sugar content considerably decreased (137). It has been found repeatedly that the application of small quantities of boric acid or borax to the soil effectively prevents or cures the trouble (18, 21, 49, 63, 74, 141). The necessary quantities range from about 4½ to 9 pounds of boric acid, or 10 to 20 pounds of borax per acre, the best time of application being at or before sowing (16, 17, 43, 106). Where the disease occurs on mildly acid soils it is associated with lack of boron compounds, but it is also found on alkaline soils which contain as much boron as should suffice for normal growth. In the latter case, it seems probable that the boron is in some way locked up and rendered unavailable for the plants. This hypothesis is strengthened by the increasing amount of heart-rot that is being found in districts where the soil is limed, pointing to a gradual withdrawal of the boron from its available condition. In England and Scotland the disease is only gradually being recognized and reported, but an increase of the trouble is anticipated if heavy liming of the sugar beet areas is carried out, as the danger of excessive calcium is already realized (13). The association of heart-rot with boron deficiency has been confirmed in controlled water culture experiments, in which similar symptoms can be induced. It is also claimed (8) that the presence of boron increases the resistance of sugar beet to poisoning by the heavy metals. Solunska (153) has examined the growth conditions which determine the effect of boron deficiency on sugar beet and suggests that water relations are of great importance. His conclusions are that the increase of moisture in the soil during the first half of the vegetative period stimulates the development of foliage and aggravates the disease, while at the very end of the vegetative period, in some cases, it may result in recovery of the plant. Fron (44) also suggests that

one of the chief factors causing heart-rot is lack of water caused by the drying out of the soil.

Brown-heart of turnips is a parallel disease which has been successfully controlled in Canada (89, 90) by the application of ten pounds per acre of borax. This has since been confirmed by experiments in Scotland and Wales (114a, 169).

Boron is now generally recognized as essential for tobacco, deficiency producing characteristic symptoms of disease (152). As generally happens, the meristematic tissues are primarily affected, the stem apices die and flowering is inhibited, the effect on roots and leaves being less marked. Diseased leaves are richer in starch and sugar than healthy ones, possibly because the disorganized phloem interferes with normal transport (146, 147). Deficiency of calcium resembles that of boron in that both produce death of the terminal bud, but calcium shortage shows first at the tips of the young leaves, whereas boron deficiency is first seen as a light green color at the base of the young leaves, followed by a general breakdown (91, 93). Although the evidence is not conclusive, some indications of an association between the absorption of boron and that of calcium have been obtained in *Vicia faba* (162).

Mes (103) states that the symptoms of deficiency are most marked when vegetative growth is strongest, as has repeatedly been found with *Vicia faba* at Rothamsted. Attempts to replace boron by manganese were ineffective, though the manganese improved the vegetative growth and the green color. Boric acid or borax in small quantities has proved effective in ameliorating this deficiency, 5 pounds per acre being adequate in some cases (94). In Sumatra for years past boron compounds have been used as fertilizers in the ordinary commercial routine, their value as a preventive of disease being fully accepted (79, 104).

The tomato, another member of the Solanaceae, also needs boron for normal growth and for the setting and development of fruit (71, 151). The boron is apparently fixed in the tissues and cannot be used repeatedly by the plant, so a constant, though minute, supply is essential throughout the period of growth. When the main growing points are killed by deficiency, the buds are stimulated to develop till the boron supply is locally exhausted, when symptoms of degeneration again appear (146, 147).

Evidence for boron requirement of cereals is less definite, pos-

sibly because the minimum requisite amount is very low, enabling normal growth to be made under conditions which would induce disease in many other species which require larger amounts (138). The range of boron content in various species has been found to be lowest in barley, rye and wheat, from .1 to .3 mg. per kgm. dry weight, and highest in tomato, tobacco, potato, beans and peas, rising to a maximum of 18 mg. per kgm. dry weight (157).

Boron deficiency is accompanied by abnormal tillering in wheat, which can develop right up to the flowering stage in solutions containing no boron (111). The ears, however, either do not emerge or are badly developed and sterile, a condition which also appears in maize under similar circumstances (122). Oats may possibly need boron, though this is not fully established, as the yield of straw has been increased by boron manuring, but the yield of grain reduced (88). The incidence of fungus disease appears to be influenced by boron supply, and Eaton (38) found that *Erysiphe graminis* was abundant on boron deficient barley plants when it was absent from those receiving boron, whereas *Helminthosporium sativum* behaved inversely, the attack increasing in severity with increasing amounts of boron in the nutrient solution.

The injurious action of boron for citrus trees is widely recognized, and sufficient is contained in some irrigation waters to damage both citrus and walnut (143). Minute quantities are, however, essential and the anatomical and physiological changes induced by boron deficiency have been reproduced in controlled experiments (50, 53, 54). Here again the meristematic tissues are primarily affected, gum formation following. Abnormal carbohydrate accumulation occurs in the leaves, but as this excess is rapidly reduced if boron is supplied, it seems probable that an improvement in the conducting tissues plays an important part in the recovery of the plant.

Symptoms of boron deficiency manifest themselves later and develop more slowly during spring and autumn than in summer. This appears to be due to the reduced length of day (161), rather than to lower temperature. A certain correlation exists between the factors of boron and length of day as, with a variety of species, in the absence of boron the influence of length of day was found to be less striking than when boron was present, whereas

the boron deficiency symptoms were less pronounced under short day than under full day conditions.

Now that the signs of boron deficiency are becoming known, growers are beginning to attribute various cases of unhealthy or failing crops to this cause, and in many cases the trouble can be overcome by the use of small dressings of boron compounds. Sugar cane in water culture experiments exhibited depressed growth, distorted and chlorotic young leaves, and definite stem and leaf lesions in the absence of boron, normal growth being resumed if as little as .22 p. p. m.[1] of boron was added to the nutrient solution (99). The demands of strawberry are somewhat greater, varying according to the season of the year, 1 p. p. m. of boron preventing deficiency symptoms in spring, but not in summer. Cases have been observed in field conditions where deficiency of boron has definitely limited growth (57). Flax soon perished without boron, showing decay of the growing points of the shoots, and bad development of lateral rootlets (148). In water cultures the dry weights obtained were

With boron	8.07 gm.	100 per cent
Without boron	.14 gm.	1.73 per cent

Under soil conditions, better growth occurred if boron was added, and the flower buds appeared earlier. Overtreatment with calcium- or manganese-carbonate on certain soils causes boron deficiency in flax, as it does with sugar beet, a condition that can be remedied by fertilizing with boron compounds (156).

Claims have also been made for the need of cotton (39), red clover (47), soybean (114), lettuce (86, 87), buckwheat (105) and blueberry (145) for boron, and doubtless other species will be added to the list as time goes on. Germination of maize and the early stages of growth in potatoes have also shown benefit from traces of boron (134). Care will be needed, though, not to attribute all obscure plant diseases to boron deficiency without adequate proof. Although it is perfectly clear that in many cases it is genuine damage due to lack of boron which is cured by the application of boron, we do not yet know whether in the soil there are other conditions causing unhealthiness in plants which are remedied

[1] Parts per million.

by treatment with boron. If this should be the case, the benefit of boron would be indirect, and not direct, but so far no evidence of this type of action has come to light, except in the possible case of rubidium injury in potatoes, which has been mitigated by the supply of available manganese and boron (56).

COPPER

For many years past the importance of copper has been recognized in connection with its function as the active principle of Bordeaux mixture, used for controlling fungal disease on important cultivated crops, as *Phytophthora infestans* on potato and *Peronospora viticola* on vines. This naturally led to enquiries as to the possible harmful action of the copper which falls to the ground during spraying, but all evidence in this respect has been negative, as the copper forms insoluble compounds in the soil with chalk, oxide of iron and alumina, and is, therefore, removed from the sphere of action (128).

More recently, the importance of copper in certain aspects of animal physiology has attracted much attention, and suggestions have been made that this element, in very minute quantities, may also be essential to plant life. This claim is by no means proved, but there are definite instances where copper has certainly improved growth in one way or another, though it cannot yet be regarded as "essential" in the true sense of the word. Far more critical work is necessary before copper can be considered on the same footing as boron and manganese.

The most spectacular work with copper was that of Allison, Bryan and Hunter (3) in 1927 on the sawgrass peat in Florida, when they found that the wholly unproductive soil could be made to produce excellent crops of lettuce, radish, turnips, rape, tomatoes, etc., by the addition of 30 pounds of copper sulphate per acre without any further manuring. Since then, various workers have found that copper is beneficial to crops grown on peat and muck soils (164), but, as in some cases similar effects can be obtained by the use of certain manures, as potash (167, 173), it is still a moot question as to whether the action of the copper is directly on the plant, or whether it acts indirectly by ameliorating some adverse condition in the soil. Allison (2) has attacked this problem and claims that in sugar cane normal development follows if copper is

introduced into the plant in other ways than by the roots, though this still does not eliminate the possibility of some action of copper on toxins absorbed from the soil. Whatever the explanation, the benefit itself cannot be questioned, and in Holland it is the recognized practice in reclamation of peat land to add 50 kg. per hectare of copper sulphate during the first year, as a preventive of what is known as "Urbarmachungskrankeit" or reclamation disease (cf. 20, 149, 171).

Certain types of chlorosis can be remedied by application of copper salts, either with the fertilizer at the roots or by spraying the leaves. The "frenching" or spotting of citrus leaves has been cured by spraying with Bordeaux mixture or by applying copper sulphate to the soil (117). Despite the increase in chlorophyll production, however, no copper was detected in the chlorophyll itself. Exanthema in pear trees is also attributed to copper deficiency, but there is no evidence as to whether the disease is due to the absence of copper *per se,* or to the presence of soil toxins, the effect of which is neutralized by the action of copper (118). Chlorosis of other deciduous fruit trees has also been cured by the use of ¼–2 pounds copper sulphate per tree, applied to the soil (5, 67). From another point of view the addition of copper sulphate to the usual fertilizers is said to improve the thickness and color of onion scales, though the results are not always consistent (77, 78).

Increased yields due to treatment with copper have been claimed in various quarters (135). Oats (19, 88), tomatoes (151), maize, sweet potatoes and beans (130) are among those mentioned and indicate the wide range of plants that apparently respond to copper, though in other cases no benefit was found with tomatoes (119) or buckwheat (105). It must, however, be remembered that an element may stimulate growth without being essential in the sense that in its absence vital aspects of growth are inhibited or seriously checked. More proof than this is needed, and certain investigators have applied more critical methods. Using water culture methods, Lipmann and McKinney (81) found that flax grew satisfactorily without copper till blossoming, but the amount of bloom was reduced and no capsules or seeds were produced. Barley also needed copper for seed formation, 1/16–1/18 p. p. m. of Cu in the nutrient solution being sufficient. Sommer (154), on the other hand, states that flax, tomatoes and sunflowers made little growth without cop-

per after the first week in nutrient solution. None of these workers has any clue to the rôle of copper, though it has been suggested that it may be auto-oxidant or catalytic in action. Among lower plants copper, as well as manganese and zinc, has been found to increase the growth of *Aspergillus flavus* and *Rhizopus nigricans*, better results being obtained from a combination of these elements than from each separately (84). The fat content was also increased but the proportion of nitrogen decreased by a very low optimum amount, the toxic limit soon being reached.

Although the claim for the essential nature of copper in the economy of plants cannot yet be substantiated, the evidence in hand is sufficiently encouraging to justify more extended investigations under strictly controlled conditions, for it is quite possible that copper may be essential for some plants or under certain conditions, and be unnecessary in other cases and for other species.

MANGANESE

The recognized importance of manganese for growth has encouraged work on the manganese content of plants, which varies considerably between species and also in a single species grown under different conditions. Lundegärdh (82) states that manganese is very slowly absorbed and that the total soil manganese has no great influence on the uptake. The addition of manganese sulphate to the soil may increase the manganese content of crops grown thereon (28), and with spinach a larger amount was found to be absorbed when the fertilizer was applied in several small dressings instead of all at once in a single treatment (108). This may imply that the sulphate remains available only for a short time and that repeated small applications enable the plants to utilize a larger proportion before the remainder becomes unavailable. The manganese content of plants grown in soils of varying acidity has been found to increase with the hydrogen ion concentration of the soil, due to a higher proportion of manganese in the soil water of acid soils (116).

Grasses vary considerably in their ability to take up manganese from the soil, the average content in one test varying from 207.5 mg. per kilogram for *Dactylis glomerata* to 78.1 mg. per kg. dry matter for *Poa pratensis*, while lucerne was lower than any grass, with only 46.6 mg. of manganese per kilogram (14). It is gen-

erally found that the proportion of manganese is higher in the leaves than in other parts of vegetables and fruits (126). Also, for any one species at any time the green leaves are always richer in manganese than etiolated, chlorotic leaves (11). The demands of species vary considerably, as is indicated by variation in the response of different crops to manganese fertilizers.

From the practical point of view the importance of manganese lies in its ability to prevent chlorosis and to increase the yield of crops. Manganese deficiency disease is usually manifested by a loss of green color, and is apt to be most marked on soils of high pH (30), rarely showing itself on acid soils. Heavy liming on some soils is, therefore, often followed by the appearance of trouble (80) due to the manganese in the soil being rendered unavailable for the plant, and where such liming is necessary for the production of certain crops the use of manganese fertilizers is essential (166).

Chlorosis due to manganese deficiency shows itself in characteristic ways. In tomato and cucumber plants the tops are first affected, the intravenal tissue of the leaves gradually changing from green to yellow while the veins and midribs remain green, producing a definitely mottled appearance. The general growth is weakened and the flower buds usually turn yellow and fall before opening (59). It appears that certain factors, as low temperature and slow growth, enable plants to withstand manganese chlorosis and that the trouble can also be overcome either by the addition of manganese compounds to the soil or by the correction of the soil reaction so as to make the manganese which is present available to plants (46). This correction can be made by increasing the acidity by the application of sulphur or ammonium sulphate, or by causing temporary water logging, in which the high degree of water saturation acts as a reducing agent (123). Many crops are improved either in health or yield by the application of manganese fertilizers, including blue lupins, soybeans (97), oats (29, 88), spinach, beets, blueberry (145), buckwheat (105), tomatoes (68, 129) and cucumbers, 100–150 lbs. per acre of manganese sulphate being effective with the latter (58). When all the tests are reviewed, however, it is evident that crops differ considerably in respect of their manganese requirements (115). In some cases, the reports

indicate that no benefit has been derived from manuring with manganese (64).

On the other hand, a type of chlorosis is induced also by excess manganese, as may occur with blue lupins on limed soil if there is a deficiency of iron (142), while poor growth of rice has been observed in the presence of an excess of soluble manganese in the soil (72). Chapman (25) has put forward the hypothesis that excess of manganese hinders the transport of iron to and from the leaf by converting it into an insoluble ferric form, thus causing chlorosis.

Citrus, sugar cane and tobacco are three other crops which react to deficiency of manganese. In Florida and California the growth of citrus and the quality of the fruit have been much improved and any tendency to chlorosis overcome on alkaline soils by applications of manganese salts (150). Haas (51) found that in acute cases of deficiency, citrus leaves absciss prematurely and the shoots die back. The roots remain healthy after deficiency symptoms are manifest in the shoots, suggesting that the roots absorb what manganese is available and retain it, not surrendering it to the stem and leaves unless more manganese is supplied.

Sugar cane exhibts the usual type of chlorosis due to manganese deficiency, and the yield and quality of the sugar is also affected (34, 98). The purity of the cane sugar is usually higher on soils with high manganese content (158), and there is also less brown-stripe disease on such soils (55). Sugar beet also shows increase in yield and sugar content if manganese deficiency is eliminated (48). The relation of sugar to manganese has been worked out in other species. Wheat, maize, lettuce and tomatoes grown without manganese have been found to be lower in reducing sugar and sucrose than those receiving manganese, indicating that the element plays some important part in sugar formation and sugar metabolism. In these experiments the manganese was injected straight into the stems, thus enabling both control and manganese plants to be grown in the same pots under identical conditions of nutrition and environment (107).

Apart from chlorosis, various other observations with relation to manganese have recently been made. Tobacco, while showing the usual deficiency chlorosis (93), is also very sensitive to excess, as plants growing on acid soils may be injured by toxic quantities

of soluble manganese (15). In such cases, phosphate fertilizers reduce the injury, probably by rendering the excess manganese inactive in the plant. Walnut yellows is a disease that is still little understood and for which manganese deficiency has been suggested as the cause. As, however, affected walnut leaves and bark contain a higher proportion of manganese than the healthy tissues, the cause cannot be attributed to manganese deficiency unless a considerable amount of the element that is present is for some reason or other unavailable for use in plant metabolism (52).

A most important aspect of manganese deficiency is its relation to grey-speck disease of oats and wheat. Inspired by Samuel and Piper (132), various investigators have obtained control of the disease by judicious use of manganese (33, 101, 163). Gerretsen, however, is now claiming that this does not represent the whole of the story, but that other factors of a bacterial nature combine with the manganese deficiency to cause the disease (45). Manganese deficiency in barley has been dealt with effectively by drilling ½ cwt. of manganese sulphate with the seed (144).

Claims have been made that small quantities of manganese stimulate the growth of various plants (136). As, however, this has been specifically claimed for plants grown under alkaline conditions it remains an open question as to whether a genuine stimulation occurs, or whether it is merely that an incipient manganese deficiency, causing reduction of yield without external symptoms of damage, is overcome by the application of manganese salts, resulting in an improvement in growth which suggests stimulation. Seeds of chickpea and peanut treated with .5 per cent solutions of manganese sulphate before sowing have been found to grow faster than control seeds for the first 10–20 days, this being attributed to the effect of the salt in accelerating enzyme activity during mobilization of food reserves and early stages of plant growth (172).

A certain amount of work has been continued with cryptogams and simpler phanerogams. Traces of manganese are beneficial to yeast, increasing the dry weight (85), though the toxic limit is soon reached, resulting in decreased growth or death of the cells. Further claims are made for the importance of manganese for *Aspergillus niger,* for which it appears to be essential for normal growth and sporulation (155). Hopkins (60, 61) found that the green alga *Chlorella* made no growth without manganese and sug-

gests that the element functions physiologically in an indirect manner by its action on the state of oxidation of iron, so that sufficient manganese must be present to ensure the oxidation. The evidence in regard to *Lemna* is conflicting, as workers who originally stated that manganese had no beneficial effect (27), have repeated their work and now state that minute traces in the nutrient salts must have been overlooked, and that it appears that *Lemna major* does need a trace, though 1 : 300,000,000 is sufficient to give good growth (26). Here again the toxic limit is very soon reached, 1 mg. Mn per litre being too high a concentration for optimum growth (131).

ZINC

The essential nature of zinc in fungus nutrition has been claimed for many years, since Raulin (125) worked in 1869 with *Aspergillus niger*. After much controversy, the general opinion is that this view is correct, and various workers have put forward further proof in the last five years. Steinberg (155) states that normal growth and sporulation of *Aspergillus niger* can occur only in the presence of several minor elements, including iron, copper, manganese and zinc. The dry weight of yeast (85) is also increased by zinc, though, as usual, too heavy doses are toxic.

Among the higher plants claims are made for the value of zinc from two aspects—as a stimulant to the growth of certain crops, and as a specific against certain diseases. McHargue and Shedd (88) obtained increases in straw and grain of oats by the addition of traces of zinc to sand cultures, whereas Scharrer and Schropp (140) found greater stimulation with other cereals and peas than with oats. Buckwheat and flax have also been improved by zinc in some circumstances as, for instance, where flax was grown on heavily limed acid soil (73).

The more important aspect is in relation to plant diseases which are attributed to a deficient supply of zinc. Frenching or mottle-leaf of citrus, little-leaf of fruit trees (96), court-noué of vines (36), pecan rosette (42) and bronzing of tung trees (113) have all been successfully treated by zinc sulphate, leading to the assumption that zinc is essential for certain metabolic functions and that a deficiency of the element hinders normal development. Applications of zinc sulphate to the soil, varying from .25 to 15 lbs. per tree, according to the type of citrus, have caused marked im-

provement in badly frenched trees (23), but this method sometimes fails. Spraying with solutions of zinc sulphate is more generally successful (159) and sometimes direct injection into the tissues is satisfactory (95). According to Dufrenoy and Reed (37) zinc, as well as iron, has a specific effect on leaf assimilation, mottle-leaf being a pathological symptom indicating an interruption of equilibrium between the cytoplasm and its inclusions. The provision of zinc to affected plants increases chlorophyll production and photosynthetic activity. A significant point is that zinc is present in the cells of treated, but not untreated, orange trees, leading to the direct association of zinc with recovery of the plant.

Little-leaf or rosette of fruit trees is characterized by the production of numbers of abnormally small leaves, and the value of zinc as a corrective is acknowledged from many quarters. Opinions differ as to the best method of application, and soil applications, injection into the tissues (35) and spraying (121) or dipping all have their advocates. While it is probable that little-leaf of fruit trees is a symptom of an inadequate supply of zinc for normal metabolism, Chandler, Hoagland and Hibberd (24) point out that the trouble may not be due to zinc deficiency only, as large woody perennials grown on the same soil as the fruit trees are also susceptible, while annual plants are generally free from attack. They suggest the possibility that zinc may aid in the precipitation of toxic substances formed by certain soil bacteria, and that the beneficial action of zinc may thus be indirect rather than direct.

Though the beneficial effect of zinc in these various types of abnormal development cannot be denied, no definite proof yet exists that zinc is essential for normal development of higher plants. Such proof can be given only by experiments in which plants are grown from seed in the entire absence of zinc, as has been done with boron, copper and manganese. If little-leaf, frenching, etc., could then be produced artificially, the practical results already available would provide a most valuable weight of evidence in support of the hypothesis.

OTHER ELEMENTS

Up to the present, definite evidence of the essential nature of "minor" elements has been established only for the four elements already examined. A considerable amount of work has been done

with others with the same objective, but so far with very little success. The much-tested fungus, *Aspergillus niger,* has shown some stimulation with very low strengths of various elements (124), higher concentration being very toxic. Other elements exhibit toxicity and among the halogens fluorine is the most poisonous. With higher plants only indifferent or toxic action has so far been proved with such elements as bromine (168), fluorine (105, 112, 133), molybdenum (139), selenium (65, 66, 76), uranium (7), thallium (62, 83, 92), caesium (4), palladium, beryllium and zirconium (6, 22). Rubidium is usually found to be toxic or indifferent (4, 22) though Loew had earlier claimed that in small amounts it benefitted Chinese cabbage, barley and spinach. Rather more evidence of occasional stimulation occurs with a few other elements, described below in more detail.

ALUMINUM

Toxicity of aluminum is repeatedly being shown (41) but there is no evidence that small quantities of the element are essential for growth. The beneficial effects occasionally recorded are generally due to soil reaction caused by the aluminum liberating supplies of definitely necessary elements, such as iron (127), of which scarcity causes chlorosis.

ARSENIC

Arsenic is also noted for its toxicity (31, 120), but in conjunction with other minor elements it has been found to increase the frost resistance of young maize plants under certain cultural conditions (122).

BARIUM, LITHIUM, STRONTIUM AND CHROMIUM

The first three may possibly play some part in the metabolism of sugar cane, as they have all been found in very productive soils, while they were absent in poor soils. Strontium, with chromium and zinc, may also exercise some inhibitory action on diseases of sugar cane, which is interesting in view of the stimulation exercised by strontium on *Aspergillus niger* in high concentrations (124). In some areas, the proportion of brown-stripe disease of sugar cane varies inversely with the amount of chromium present in the soil (55).

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GENETICS OF POLYPLOIDY*

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Changes in chromosome number directly affect gene inheritance and, in turn, the morphology and physiology of cells and of plants. Were additional proof of the gene-chromosome law of heredity necessary, the genetical behavior of polyploids affords a most potent experimental verification, for with a change only in chromosome number, a direct, corresponding change in inheritance is made manifest.

For the general botanist, changes in chromosome number may conveniently be reviewed in their results on (1) gene and character inheritance and (2) general effects on cell and plant morphology, physiology, ecology and evolution. Since a detailed account of the cytogenetical aspects of polyploidy may be found in Sansome and Philp's book (45) on "Recent Advances in Genetics," in Darlington's "Recent Advances in Cytology" (15) and in Sharp's last edition of "Introduction to Cytology" (46), only the broader and newer points will be developed in this review.

The following brief glossary of terms will probably be of assistance to the lay reader.

1. Change in chromosome number in only one pair of the diploid.
   - Monosomic $= 2n - 1$ (also called haplo-forms).
   - Trisomic $= 2n + 1$ extra chromosome.
     - Primary $= 2n + 1$ (extra is complete homologue of one pair).
     - Secondary $= 2n + 1$ (extra has two similar arms).
   - Tetrasomic $= 2n + 2$ (both extras homologous with one original pair).
     - Gene conditions—Quadruplex *AAAA*, triplex *AAAa*, duplex *AAaa*, simplex *Aaaa*, nulliplex *aaaa*.
2. Change in chromosome number of all sets or pairs.
   - Heteroploid (polyploid)—a form with chromosome number other than the true haploid (monoploid) or diploid number.
     - Euploid—an exact multiple of the haploid (triploid, tetraploid, penta-, hexa-, hepta-, octoploid, etc.).
       - Autoheteroploid (autopolyploid)—a multiple chromosome complement of a single kind of the haploid set. An autotetraploid, for example, has four similar chromosomes in each set.
       - Alloheteroploid (allopolyploid)—a multiple chromosome complement of dissimilar sets of chromosomes.
     - Aneuploid—a chromosome number other than an exact multiple of the haploid.
       - Hypoploid—a little lower than some multiple.
       - Hyperploid—a little higher than some multiple.

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Strictly speaking, monosomics and even trisomics should not, perhaps, be included under the term polyploidy but some of their cytogenetical reactions, especially of the trisomics, often prove useful in understanding the more complex behavior of the true polyploids.

Gene Segregation in Polyploids

Gene distribution is directly affected by the chromosome behavior in triple (trisomics or triploids), quadruple (tetrasomics or tetraploids) or in the higher multiple sets, so that different genetic ratios emerge from hybridization experiments. Chromosome behavior in triploids is reasonably similar in most species, but in tetraploids there are differences in disjunction among the various species that influence fertility (4, 5, 16, 22, 24, 32, 37, 40, 49).

Gene Ratios in the Triple Condition

Triploids ordinarily arise from the union of gametes containing $2n$ and n chromosomes. They have been produced experimentally by crossing tetraploids and diploids, better success usually following use of the $4n$ as the maternal parent. In the reciprocal cross, $2n$ pollen tubes of the tetraploid are not particularly adapted to germination or growth in the $2n$ stylar tissue.

Meiotic divisions in triploids are very irregular. Sometimes trivalents are formed but very frequently a bivalent and a univalent are observed. In either case, unbalance of chromosomes occurs and the percentage of abortive gametes is high. The functional gametes of a triploid are usually those containing the n, $2n$ or the $n+1$ and $n+2$ number of chromosomes. The progeny, likewise, show a high frequency of $2n+1$ forms plus a small percentage of tetraploids. The characteristic sterility of triploids seems largely to be due to the unbalance of genes in the gametes.

Because of the great amount of sterility in triploids it is not profitable to discuss genic inheritance. Better results come from a study of trisomics where only one chromosome at a time is added.

The classical research with *Datura* trisomics (5, 6, 8) is so well described in text books that details will be omitted in this review. The same is true for the well known *Drosophila* investigations of trisomics, as well as those in maize (34, 41, 42), tomato (28, 29, 30) and *Nicotiana* (12).

Trisomics ordinarily arise from triploids or from diploids through non-disjunction of one bivalent. With three homologous chromosomes, X, X' and x, random pairing should give the gametes XX' Xx ,$X'x$, X, X' and x. In a duplex trisomic (AAa), the gametic ratio of genes should be $1AA:2Aa:2A:1a$, whereas in a simplex form (Aaa) the ratio becomes $2Aa:1aa:1A:2a$. But these ratios are never realized in breeding experiments because the male gametes with the extra chromosome are rarely functional in competition with normal microspores. Even the female gametes with the extra chromosome are usually represented much less frequently than 50 per cent.

Cytologically, chromosome disjunction of a trisomic is typically a random 2×1 affair, and only two of the chromosomes synapse or crossover at any one region. Hence, some of the progeny of a self-fertilized plant are disomic and some trisomic. For example, in primary *Datura* (6, 8) trisomics an average of only 24 per cent of the progeny is trisomic (range from 11 to 32 per cent). When once the percentage of trisomics in the progeny is determined, it is usually discovered that gene segregation follows random assortment of the three chromosomes, modified by any crossing-over in the six strand (chromatid) stage. Accordingly, the gene ratio is affected by the distance of the genes from the spindle-fiber-attachment. The recent work with maize trisomics is particularly in accord with this concept (34, 42).

Gene Ratios in the Quadruple Condition

More regular genetic data are often obtained from tetraploids than triploids due to a better balance of the chromosomes and, hence, a higher degree of fertility. In many tetraploids there is a high frequency of bivalent chromosome association at diakinesis, although some sterility enters whenever a 3×1 disjunction occurs. It is for this reason that the fertile progeny of self-fertilized tetraploids consists largely of $4n$ plants and, hence, genetic data from $4n$ hybrids can be utilized to trace chromosome and gene distribution. This is particularly true of some tomato tetraploids (32), but with maize tetraploids there are greater cytological irregularities (40).

Tetraploids arise either naturally or artificially. They may come from the occasional $2n$ gametes of triploids. Doubling may also occur in somatic tissue of the diploids. Artificially, they are easily

made in certain species of the *Solanaceae,* like the tomato, by the decapitation technique (24, 31, 32, 53) where somatic doubling of nuclei takes place in callus tissue. Such tetraploids are valuable for controlled experiments involving known genetic constitutions.

Whenever doubling occurs, two extreme situations may be encountered. In one, the chromosomes may be completely homologous or nearly so. This is the autotetraploid. An extreme case of this is the $4n$ form of *Lycopersicum esculentum* derived from the haploid *via* the diploid (31, 32). This is an absolutely homozygous tetraploid, the four chromosomes in each of the 12 sets being identical since each single chromosome came from the haploid originally. This tetraploid is the most sterile of all tomato tetraploids. Autotetraploid *Datura* plants, on the other hand, are reasonably fertile, despite the fact that their chromosomes associate as quadrivalents, allowing irregular disjunction (4, 5, 9).

The other extreme is that of the allotetraploid where two of the chromosomes in any set are very different from the other two. As a result, there is a minimum of quadrivalent prophase association and, subsequently, a high degree of bivalent chromosome pairing at diakinesis, giving fairly regular disjunction which results in a large percentage of functional diploid gametes. In most allotetraploids, however, there is some degree of quadrivalent association of the chromosomes.

Allotetraploids are more fertile than autotetraploids. In the latter, only autosyndetic pairing of similar chromosomes is to be expected whereas in allotetraploids both autosyndesis and allosyndesis (pairing of dissimilar chromosomes) may occur, depending on the phylogenetic relations of the plants involved in the formation of the tetraploid. In an allotetraploid derived from two species, such chromosome similarity, as evidenced by pairing, may serve as a basic factor in determining taxonomic relationships. In extreme cases of allotetraploidy, where no allosyndesis occurs, the conclusion that the two forms are true, legitimate, genetic species, seems to be justified.

Genetically, tetraploids (or tetrasomics) may consist of the following genotypes: *AAAA, AAAa, AAaa, Aaaa,* or *aaaa,* the letters representing genes in any one set of chromosomes. In many respects the duplex condition, *AAaa,* serves well to test the gene-chromosome relations.

Given four homologous chromosomes in any one set such as may be found in an autotetraploid or an allotetraploid where the genetic differences are not too great, there are four possible fundamental methods of synapsis and subsequent disjunction. When measured by genetic tests of $4n$ hybrids, such as *AAaa,* certain $2n$ gametes and $4n$ progeny result which may be determined by F_2 or back-cross (to recessive $4n$ types, since a $4n \times 2n$ cross is ordinarily sterile) experiments. The four methods are illustrated in Table 1:

TABLE 1

Summary of four possible methods of chromosome behavior in tetraploids

Method of pairing	Bivalent association	2n Gametes			F_2 ratio	Back-cross ratio
		AA	*Aa*	*aa*	*A* : *a*	*A* : *a*
1. Preferential (autosyndesis)	$\frac{A}{A}$ $\frac{a}{a}$	0	1	0	1:0	1:0
2. Preferential (allosyndesis)	$\frac{A}{a}$ $\frac{A}{a}$; $\frac{A}{a}$ $\frac{a}{A}$	1	2	1	15:1	3:1
3. Random assortment of 4 chromosomes ...	$\frac{A}{A}$ $\frac{a}{a}$; $\frac{A}{a}$ $\frac{A}{a}$; $\frac{A}{a}$ $\frac{a}{A}$	1	4	1	35:1	5:1
4. Random assortment of 8 chromatids	See Table 3	3	8	3	20.8:1	3.7:1

Method 1. Preferential pairing of similar chromosomes (autosyndesis). When two chromosomes of a tetrasome are very different genetically from the other two, or when a true allotetraploid is involved, and pairing is conditioned by gene-by-gene attraction, it is apparent that the two similar chromosomes should synapse. If, then, disjunction is from a bivalent condition, as is often the case, *all* the diploid gametes should be alike (*Aa*) and the hybrid should breed true. Such is rarely the case, but it has been reported in tetraploids from very wide species or genus crosses, although the evidence rests on genetic, rather than cytological, grounds. For example, in the allotetraploid *Raphanus-Brassica* hybrids (25, 26), in *Spartina Townsendii* (23) and in *Primula kewensis* (37, 39),

chromosome pairing is largely in the bivalent condition and these hybrids are very fertile and breed approximately true. In the latter hybrid, the parental *P. verticillata* chromosomes generally pair among themselves as do the *P. floribunda* chromosomes. However, one quadrivalent set is often present, indicating a certain relationship between the two species. A similar situation is found in the species tetraploid *Nicotiana digluta* (11) which arose from 12 *N. glutinosa* and 24 *N. tabacum* chromosomes that were doubled, giving 36 pairs and making a true breeding form with regular meiotic behavior.

. The above method is not found in true autotetraploids, even when the two chromosomes are markedly different genetically from the other two. Here there is quadrivalent prophase association with crossing-over between the four chromosomes (or eight chromatids) allowing for chromosome or chromatid segregation.

Method 2. Preferential pairing of dissimilar chromosomes (allosyndesis). There seems to be no apparent reason for such a condition if pairing is instituted by a gene-by-gene attraction. However, some earlier data on *Primula* (20) tetraploids were fitted to such an hypothesis, resulting in 15:1 F_2 or 3:1 back-cross ratios of dominant to recessive types. Later these meager data were better explained on another basis (method 3 below) by Muller (35).

Method 3. Random assortment of four chromosomes. If, in a 4*n* *AAaa* hybrid, the four chromosomes of any one set synapse at prophase in a tetravalent condition and later emerge as bivalents, a $1AA + 4Aa + 1aa$ assortment of diploid gametes results. This gives a 35:1 F_2 phenotypic ratio or a 5:1 back-cross ratio of dominants to recessives (Table 2). Practically all tetraploid data thus far reported have been fitted to such an hypothesis. The early *Datura* experiments (9) seem to afford a close fit to expectation for the *Pp* (stem color) genes and to a lesser extent for the *Ss* (spiny capsule) genes. In the *Primula sinensis* tetraploid the data fit closely to this method (49, 54).

Method 4. Random assortment of eight chromatids. Modern cytological and genetical research has demonstrated beyond reasonable doubt that in a diploid the two homologous chromosomes pair in early prophase and then undergo a longitudinal split, giving four chromatids. It is at this stage that crossing-over occurs.

TABLE 2

Tetrasomic inheritance in hybrid progenies, selfed and back-crossed to recessive

4n hybrids	Mated with	Progeny					Ratio
		AAAA	*AAAa*	*AAaa*	*Aaaa*	*aaaa*	*A* : *a*
I. With random assortment of 4 chromosomes							
AAAa	Self	1	2	1			1:0
	aaaa			1	1		1:0
AAaa	Self	1	8	18	8	1	35:1
	aaaa			1	4	1	5:1
Aaaa	Self			1	2	1	3:1
	aaaa				1	1	1:1
II. With random assortment of 8 chromatids							
AAAa	Self	225	360	174	24	1	783:1
	aaaa			15	12	1	27:1
AAaa	Self	9	48	82	48	9	21:1
	aaaa			3	8	3	11:3
Aaaa	Self	1	24	174	360	225	559:225
	aaaa			1	12	15	13:15

Undoubtedly, the same thing prevails in tetraploids where the eight chromatids of the four chromosomes in any tetrasomic set could be associated at prophase. Crossing-over between chromatids occurs as in the diploid. If any one gene locus is far enough removed from the spindle-fiber-attachment, such crossing-over may affect every such gene locus among the eight chromatids. This would approach a random interchange among the eight chromatids, the maximum state being a wholly random assortment of the eight. If a gene is borne near the spindle-fiber-attachment there would be less chance of an interchange separating sister chromatids. In this case, the genes would be assorted as in Method 3.

Mathematically, a random assortment of eight chromatids in a $4n$ hybrid, *AAaa,* results in a $3AA + 8Aa + 3aa$ proportion of diploid gametes (Table 3). This gives a 20.8 : 1 F_2 or an 11 : 3 back-cross ratio of dominants to recessives as a maximum. With less crossing-over, such as would hold for genes nearer the spindle-fiber-attachment, an approach to the 35 : 1 F_2 ratio as a limit would result. In other words, the percentage of recessive gametes from an *AAaa* form varies between 16.7 and 21.4 per cent, depending upon the amount of crossing-over between the spindle-fiber-attachment and the gene in question.

TABLE 3

Gene relations under complete random assortment of eight chromatids in a tetrasomic set

Bivalents	Summary of daughter cell constitution	Gametes (2*n*)		
		AA	*Aa*	*aa*
1 .... $\frac{AA}{AA}\ \frac{aa}{aa}$				
	1 *AA–AA*	1		
1 .... $\frac{AA}{aa}\ \frac{aa}{AA}$				
	16 *AA–Aa*	8	8	
1 .... $\frac{AA}{aa}\ \frac{AA}{aa}$				
	12 *AA–aa*		12	
8 .... $\frac{AA}{Aa}\ \frac{Aa}{aa}$				
	24 *Aa–Aa*	6	12	6
8 .... $\frac{AA}{Aa}\ \frac{aa}{Aa}$				
	16 *Aa–aa*		8	8
8 .... $\frac{AA}{aa}\ \frac{Aa}{Aa}$				
	1 *aa–aa*			1
8 .... $\frac{Aa}{Aa}\ \frac{Aa}{Aa}$				
Total		15	40	15
Ratio		3	8	3

There are only a few recent experiments that have been fitted to such an hypothesis. These deal with tetraploids in *Lycopersicum* (32, 44) and *Rubus* (14). For illustration, a summary of the tomato data appears in Table 4.

Each of the gene ratios in Table 4, representing six of the twelve different chromosomes of the tomato, shows a distinct tendency to be less than 35:1 in F_2 progenies. This is equally true of the autotetraploid, *L. esculentum,* and the allotetraploid represented by the species cross, *L. esculentum* × *L. pimpinellifolium.* The consistency of the data affords strong evidence against a mere random assortment of the four chromosomes and points to a chromatid assortment. The latter is apparently made variable by the amount of crossing-over between the spindle-fiber-attachment and the gene in question. For example, the *Yy* genes approach most closely the 35:1 ratio of chromosome assortment, indicating that these genes are close to the fiber-attachment. The *Rr* genes approach very

TABLE 4

Tetraploid F_2 segregations of genes carried on six different chromosomes

Tetraploid, duplex hybrids	*D*	*d*	*R*	*r*	*Y*	*y*	*C*	*c*	*A*	*a*	*B*	*b*
L. esculentum (Sansome, 1933)	995	43	647	26	554	17			172	4	207	8
L. pimpinellifolium × *L. esculentum* (Lindstrom and Humphrey, 1933)	883	28	376	19	382	13	545	33				
New data—1935	772	37	165	10			769	59	992	37		
Total	6250	108	1188	55	936	30	1314	92	1164	41	207	8
Ratio	25	1	22	1	31	1	14	1	28	1	26	1

closely the 21 : 1 ratio of a wholly random assortment of eight chromatids, indicating that they are farther removed from the attachment. The *Cc* genes in Table 4 appear to favor an allosyndetic (Method 2) pairing, but they should not be considered as critical evidence because of the uncertainty of classification of the characters involved (tomato *vs.* potato leaf) in the young plant stage when some of the counts were made. With this exception, the $4n$ tomato data seem to follow a cellular mechanism of gene distribution that is based on chromatid segregation, modified by some degree of crossing-over.

It is worth pointing out that the autotraploid tomato hybrids in Table 4 give approximately the same genetic results as the more allotetraploid forms produced by crossing the wild and the domesticated species. In this latter allotetraploid form with 48 chromosomes, two chromosomes in each set of four are identical *esculentum,* and two are identical *pimpinellifolium* chromosomes. This is true because the tetraploid arose from a $2n$ F_1 hybrid of the two species in which there were twelve pairing sets of chromosomes. The doubling of the $2n$ F_1 was done artificially under controlled conditions (32). Thus, in any of the 12 tetrasomes there are two wholly identical *esculentum* and two identical *pimpinellifolium* chromosomes with every gene alike among the two, and with hundreds of allelomorphic differences between the genes of the two species.

It should be noted, however, that there must be many more genic similarities than dissimilarities in the two species making up the

tomato allotetraploid because all the chromosomes of the diploid F_1 species-cross synapse, albeit incompletely. The same homology is undoubtedly carried over into the tetraploid hybrid. Hence, this partially allotetraploid form undergoes a chromosome synapsis similar to the autotetraploid, resulting in a corresponding genetic behavior.

An interesting and critical point in differentiating between chromatid and chromosome segregation in tetraploids arises in observing the genetic behavior of a triplex condition of the genes, such as *AAAa*. If these genes followed chromosome segregation (Method 3), the bivalent gametes could only be *AA* or*Aa,* and no recessive character would emerge in the progeny. But with chromatid segregation, three kinds of gametes are possible, *AA, Aa* and *aa* in the proportion of 15: 12: 1 (Table 2).

Special cases of polyploid genetics, due to peculiar cytological conditions, may arise to give ratios intermediate to those shown in Table 4. A recent example of this is the tetraploid hybrid of maize and perennial teosinte (13, 17, 18). In one such case (13) the hybrid had 20 maize and 20 teosinte chromosomes, giving regular meiosis and good pollen. A much smaller percentage of recessive (waxy) gametes was found than the 16.7 per cent expected with random assortment of four chromosomes or the 21.4 per cent due to random assortment of eight chromatids. This was accounted for by a greater autosyndetic than allosyndetic pairing. A formula was devised for measuring the degree of autosyndesis as follows:

$$t=\frac{1-6x}{1-2x}$$

where x = the ratio of recessive gametes and t = the 'coefficient of autosyndesis.' From this formula a t of -1 means complete allosyndesis, $t=0$ means random pairing and $t=+1$ means complete autosyndesis. Collins and Longley (13) found values of .90 in F_1, .77 in F_2 and .74 in F_3 material, all indicating a high degree of autosyndesis for the waxy gene-containing chromosomes. Evidently, the maize chromosomes pair preferentially in this genus cross. Genetically the same situation was reported by Emerson (17) who had strong evidence that the teosinte chromosomes of a maize-teosinte hybrid paired preferentially.

Gene Ratios in Higher Polyploid Cases

There is no critical genetic research with the higher polyploids. In the octoploid *Dahlia* case (27) Lawrence has reported on two sets of genes but both really show tetraploid inheritance.

Linkage in Polyploids

Several cases of linked inheritance have been reported in polyploids (44, 54), but the complexities of the situation render the subject a highly technical one which has no place in a general review. De Winton and Haldane (54) report that the linkage intensity in $4n$ *Primula sinensis* is approximately the same as in the diploid form. Sansome found the same condition in the tomato (44). For reference, Haldane's (21) statistical treatment of theoretical linkage intensities will serve the specialist.

Inbreeding and Random Mating in Polyploids

The genetical behavior of polyploids in later generations, following various systems of mating, is reported in two papers, one by Haldane (21) and the other by Bartlett and Haldane (2). In the first paper, the gametic series to be expected from various types of heterozygous autopolyploids are listed, together with the effects of self-fertilization and random mating on populations. In the second paper, formulae and rates of decrease of heterozygosis under brother and sister matings are given.

General Effects of Polyploidy

Increase in chromosome number affects the organism in several general respects. Cell size is directly modified as may be seen by a typical set of polyploid data of the tomato (Table 5). Cell volume is approximately doubled or slightly less than doubled, with chromosome doubling. In *Funaria* tetraploids, Wettstein (51, 52) reports that cell volume increased at least 1.7 times that of corresponding diploids.

It is well known that plant size is also affected. The effect of n, $2n$ and $4n$ chromosomes on tomato size is well illustrated in Figure 1 which is a typical case and is particularly convincing because the doubling was done asexually under complete experimental control (decapitation technique).

TABLE 5

Linear micromorphological measurements of tomato polyploids in microns

From Lindstrom and Humphrey (32)

	Pollen diameter	Cell size Diakinesis	Nuclear size Diakinesis
L. esculentum—n	25.7	13.4	7.7
L. esculentum—2n	25.7	16.5	9.0
L. esculentum—4n	30.0	21.8	13.1
L. pimpinellifolium—2n	21.6	16.7	8.9
L. pimpinellifolium—4n	27.1	21.9	12.2

Cell volumes of tomato polyploids in cubic microns

From Humphrey (22)

	Pollen	Pollen mother cell at Diakinesis	Nucleus at Diakinesis	Nucleolus at Diakinesis
n	8693	1251	228	3.6
2n	8693	2356	379	8.1
4n	14040	5289	899	14.0

In *Datura* and maize trisomics, experienced observers can detect the phenotypic variations caused by each extra chromosome. Evidently each chromosome carries genes that influence the gross morphology in plus and minus directions. Another influence of extra chromosomes may be seen in the histological study of *Datura* trisomics (7, 47). Here the addition of any one of the 12 chromosomes had a detectable effect on the anatomical pattern of petiole structure.

Sexual differentiation due to tetraploidy has been reported in *Sphaerocarpus* (1) where spore dyads (instead of the regular tetrads) give rise to diploid gametophytes. These are functionally females although containing both *X* and *Y* chromosomes.

Physiologically, there are striking effects of polyploidy on the plant as a whole. Growth rate is usually slowed down, the tetraploid being slower than the diploid and the diploid slower than the haploid. Regeneration (from cuttings or from callus) is, likewise, faster in the haploid than the diploid.

In this connection it is worth noting that in some species there seems to be a relationship between polyploidy and growth habit.

The diploid (20 chromosomes) *Euchlaena mexicana* is an annual, while the tetraploid *E. perennis* (40 chromosomes) is a perennial. Longley (33) has shown that seven annual species of *Sorghum* have 10 as the haploid number whereas the perennial species, *S. halepensis,* contains 20 chromosomes and a closely related genus, *Sorghastrum* with 20, is also perennial.

Differential ecological relations in polyploid series have recently been reported in *Dianthus* (43). In this genus Rohweder finds that diploid species possess a more limited longevity and poorer adaptability than tetraploid or hexaploid forms. Tetraploid carnations were found to surpass considerably the diploids in their adaptation to lime. The forms with larger chromosomes withstood poor soil conditions better than those with small chromosomes.

The real causes for these general increases in cell or plant size and in physiological response are not fully understood. Critical research on these points is highly desirable, particularly in differentiating between the action of the genes themselves and the gene chromatin. In a tetraploid, for example, not only are the genes doubled but also the chromatin. The latter substance is largely composed of chromomeres which certainly differ visibly in size. The specific size of a chromomere may well be considered as the result of the relative biochemical (catalytic) activity of the contained gene. If so, there may be a certain mass action of the gene chromatin itself which could easily be reflected in subsequent development.

Accordingly, an increase of chromosome number in polyploids attended by an increase in cell and plant size or by a slower rate of growth and regeneration, might well be the result of the added chromatin, in addition to any direct effect of the genes themselves. It is reasonably possible that the chromomere granules (or bands) are local accumulations of material synthesized by the genes and that the specific size of the chromatin granule reflects the biochemical activity of the gene.

Evidence on this very important point is still lacking but certain leads in this direction are available. It has been shown that chromosome size (and hence chromatin mass) in the larger domesticated tomato species is 30 per cent greater than that of the smaller and earlier wild form (32). The two forms presumably have approximately the same general linear arrangement of gene loci

because pairing in the F_1 is fairly complete for all twelve chromosome pairs. It is possible that the increased size of the domestic form is at least partially due to its greater chromatin mass. This hypothesis would not, of course, invalidate modern genetic findings on the particulate nature of linkage relations of size genes since the chromomere chromatin itself is linearly distributed on the chromosomes in a discontinuous manner.

Whether or not a generalization could be made that the usual increased size of domesticated races is partly due to increased chromatin mass is of course very debatable. For example, there is no assurance that the domesticated tomato arose from the present wild species. But it is interesting to note that Rohweder's (43) measurements of *Dianthus* chromosomes show much the same condition

TABLE 6

Micromorphological data on Dianthus species in microns

(From Rohweder, 1934)

	Chromosome diameter	Chromosome volume	Nuclear diameter
15-chromosome species			
Armeria	.28	.38	8.0
deltoides	.37	.79	8.1
pruinosus	.38	.83	10.1
neglectus	.39	.93	9.0
alpinus	.44	1.33	8.3
glacialis	.46	1.53	8.7
pinifolius	.48	1.75	7.8
graniticus	.49	1.92	8.8
sylvestris	.64	4.08	9.0
Carthusianorum	.73	6.10	9.5
barbatus	.80	8.20	8.3
superbus	.83	9.00	10.0
30-chromosome species			
Sinensis	.37	1.6	10.4
saxifraga	.41	2.2	9.3
orbelicus	.53	4.7	9.3
subacaulis	.55	5.1	9.6
serotinus	.67	9.3	9.9
arenarius	.75	13.4	11.3
Sternbergii	.76	13.9	11.5
45-chromosome species			
Seguieri	.36	2.1	11.6
caryophyllus	.52	2.1	7.0
plumarius	.63	11.8	10.4
monspessulanus	.73	18.4	12.6

as may be noted in Table 6. The cultivated species, such as *D. barbatus, D. superbus* and *D. caryophyllus,* certainly have a significantly larger chromatin size than their wilder relatives.

In his monograph on *Narcissus,* Fernandes (19) demonstrates clearly the size differences of the chromosomes in the hybrid between *N. reflexus* and *N. bulbocodium.* In his Figure 7, it is evident that the phenotypically larger species is *N. reflexus* which also has the larger chromosomes. Chromatin size differences in the same F_1 plant have also been reported in *Aesculus* (48) but not correlated with the phenotype.

While the above-mentioned evidence is not all taken from polyploid material, it does suggest that chromatin mass is directly related to phenotypic development. Accordingly, the greater chromatin mass of polyploids may well be responsible for some of the effects of increased size, but certainly not for all because in many forms neither additional chromatin size nor chromosome number has such an effect. In this case, the influence of the genes themselves outweighs that of the chromatin.

Evolutionary Significance of Genes in Polyploids

Perhaps the most important problem in evolutionary genetics is concerned with the origin of new genes. Certainly, there is no experimental evidence that genes arise *de novo,* and yet it is reasonably to be expected that highly complex forms of life have more and different genes than simple forms. To some extent, the extreme developments of domestication would also seem to call for some new genes, not necessarily in any great number, however.

Polyploidy offers a partial explanation for the emergence of new genes in a narrow sense. Whenever chromosomes are doubled, and later begin to act as bivalents instead of quadrivalents, a whole new series of loci is available for gene mutations in different directions from their former sister loci. Surely the less specialized genes could then serve as new points of departure by the mutation process.

It is a well established fact that the plant kingdom has been subjected to chromosome doubling or multiplication. The following recent table from Wanscher (50), giving the chromosome numbers of 3326 species, shows strikingly distinct modal numbers at 4, 8, 12, 14, 16, 20 and 24 chromosome pairs (Table 7). From

TABLE 7

Chromosome numbers in species of angiosperms

(From Wanscher, 1934)

Chromosome pairs	Monocots	Dicots	Total species
3	5	2	7
4	16	34	50
5	8	28	36
6	23	115	138
7	143	290	433
8	56	442	498
9	43	155	198
10	59	106	165
11	26	201	227
12	67	327	394
13	13	75	88
14	92	174	266
15	8	46	54
16	28	135	163
17	15	81	96
18	27	56	83
19	5	45	50
20	50	61	111
21	48	31	79
22	12	38	50
23	2	13	15
24	18	107	125
Total	764	2562	3326

such data as these, it is beyond any reasonable doubt that polyploidy must have been a potent factor in species development among plants. For greater details the reader may well consult the very recent review by Brink (10) on "Cytogenetic evolutionary processes in plants."

Conclusion

In general, then, genetic evidence from polyploids harmonizes surprisingly well with concepts based on the modern gene-chromosome law of heredity. This is true for both the individual hereditary characters and the organism as a whole. With the former, it is evident that character inheritance follows the particular gene distribution even when the cytological mechanism is disturbed by the addition of chromosomes.

The organism as a whole is also influenced by polyploidy but the relations of the parts are, nevertheless, maintained. The addition

of one chromosome in a trisomic, for example, alters many individual characters and upsets the favorable balance of plus and minus factors established in the diploid by long continued selection. Nevertheless, the plant continues to function as a whole. This can mean only that there is a high degree of elasticity in an organism, affording a margin of safety for variable conditions. This may well explain the success of the mutation theory of evolution in giving new mutations time to become established and to become fitted into the germinal complex in which they arose. True polyploidy affords, in addition, extra gene loci as sources for new mutations. Such extra loci, as they mutate, must preserve a correlated function with their original sister loci and the polyploid condition would seem to afford time and protection for this process.

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GLOSSARY

By the editors

allopolyploid: a polyploid possessing unidentical sets of chromosomes derived from two or more plants of dissimilar origin.—Aase.
allosyndesis: the pairing in a polyploid of chromosomes derived from opposite parents; particularly as opposed to autosyndesis in a hybrid between allopolyploids.—Darlington.
autosyndesis: the pairing in a polyploid of chromosomes derived from the same parent; particularly its exceptional occurrence in an allopolyploid.—Darlington.
bivalent: during the first meiotic division, chromosomes appear singly (univalents) or in homologous groups of two (bivalents), threes (trivalents), etc.
chromatid: a longitudinal half of a chromosome.
chromomeres: minute subdivisions of chromatin arranged in a linear, bead-like manner on the chromosome.
cross-over: the exchange of corresponding segments between corresponding chromatids of different chromosomes.—Darlington.
diakinesis: the last stage in the prophase of meiosis, immediately before the disappearance of the nuclear membrane.—Darlington.
disjunction: the separation of homologous chromosomes during meiosis.
genotype: the kind or type of the hereditary properties of an organism.—Darlington.
homologous chromosomes: the paternal and maternal chromatin elements which bear factors affecting the same characters.
homozygous: possessing identical genes with respect to some character.
meiosis: a form of cell division in which the nucleus divides twice and the chromosomes once, resulting in a reduction in the number of chromosomes.
non-disjunction: failure of separation of paired homologous chromosomes during meiosis, resulting in their both entering the same daughter nucleus.
phenotype: the external appearance produced by the reaction of an organism of a given genotype with a given environment.—Darlington.
polyploid: an organism with more than two sets of homologous chromosomes; triploid ($3n$), pentaploid ($5n$), hexaploid ($6n$), etc.
prophase: an early stage of nuclear division.
synapsis: pairing of homologous chromosomes during nuclear division.
trivalent: see bivalent.
univalent: see bivalent.

ERRATA

CORRECTIONS WHICH SHOULD BE MADE IN VOLUME 1 OF 1935

Page 18 line 2: *Rösel, von Rosenhof* should read *Rösel von Rosenhof.*
" 18 " 28: *1924* should read *1894.*
" 62 " 19: Delete *of other films.*
" 63 " 14: *Impossible* should read *improbable.*
" 66 " 28: (*Fig. 3D*) should read (*Fig. 4*).
" 68 " 1–5: Omit these lines.
" 76 " 16: Omit (*350 x*).
" 80 " 38: *Cardinal* should read *carinal.*
" 110 " 34: *14-chromosome* should read *24-chromosome.*
" 274 " 12: *or* should read *of.*
" 274 " 36: should read *2 female, 2 male gametophytes,* etc.
" 283 " 12: *irregularities* should read *meiotic irregularities.*
" 287 " 24: should read *aneuploid plant has one or more incomplete sets of chromosomes.*
" 358 " 12: *Lipmäe* should read *Lippmaä.*
" 376 " 38: *Lipmäa* should read *Lippmäa.*
" 388 " 39: *like Bower* should read *unlike Bower.*
" 392 " 26: *27 mm.* should read *27 cm.*
" 393 " 20: *B. Dusliana supports* should read *B. Dusliana hardly supports.*
" 393 " 31: *the Yeringian rocks* should read *the supposedly Yeringian rocks.*
" 394 " 27: *Gantheliophorus* should read *Cantheliophorus.*
" 394 " 32: *Gantheliophorus* should read *Cantheliophorus.*
" 399 " 15: *triarch or* should read *triarch and.*
" 399 " 24: *no leaf-scars* should read *no leaf-traces.*
" 417 " 34: *Oppenheim's* should read *Oppenheimer's.*